Effect of Bacterial Infection on Speed and Horizontal Trajectory of Circumnutation in Bean Shoots

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ABSTRACT

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Circumnutation movements of *Phaseolus vulgaris* 'Kentucky Wonder' are inhibited by either of the two bacterial pathogens *Xanthomonas campestris* pv. *phaseoli* or *Corynebacterium flaccumfaciens*. Pattern of the horizontal trajectory and speed of the shoot tip are influenced by length of the free-moving shoot. In infected plants, length of the shoot is often reduced, along with the speed of the tip. However, when shoots of healthy and infected plants are of equal length, speed is slower in infected plants. Results from these studies suggest that effects of the two bacterial pathogens on circumnutation movements of bean shoots involve more than a reduction in shoot length.

Additional key words: oscillation, plant movement, rhythm.

Many plants, including cultivars of beans (*Phaseolus* sp.), "climb" by winding around poles, wires, and other objects. These rotations of plant organs (circumnutations) result from a more or less rhythmic extension rate recurring on different sides of an organ (2). Stem movements have been examined in many species (1), and the phenomenon has been the subject of various hypotheses and models (2,5). These models include analyses at the molecular as well as the whole-plant level. Circumnutations play an important role in both the structural (cells, tissues, and organs) and temporal (rhythmic) organization of vascular plants (4,5).

Following a precedent established in human pathology (6), our research emphasizes a temporal component of the diseased plant. More specifically, our objective was to examine the effects of two bacterial pathogens on the speed and configuration of rotations (circumnutations) of the shoot tips of diseased bean plants.

MATERIALS AND METHODS

Seeds of *Phaseolus vulgaris* L. 'Kentucky Wonder' were planted in 11-cm-diameter clay pots containing a 1:1 mix of topsoil and sand. After emergence, plants were selected for uniformity and thinned to one per pot. Plants were maintained in a greenhouse with supplemental lighting to provide a light/dark cycle of 15/9 hr; temperatures ranged from 22 to 28 C. Ten days after planting, when plants had expanded unifoliolate leaves, each plant was inoculated with one of two bacterial pathogens: *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye (*X. c.* pv. *phaseoli*) or *Corynebacterium flaccumfaciens* (Hedges) Dawson. Plants were inoculated with a scalpel dipped in a 24- to 48-hr-old culture on Kado's medium (3). The scalpel point was inserted halfway through the stem on each side of the stem between the cotyledonary and first-leaf nodes. The same procedure without bacteria was followed for the uninoculated (control) plants. When symptoms appeared, plants were placed in a controlled-environment chamber maintained at 26 ± 1 C on a light/dark cycle of 12/12 hr providing 145 $\mu E \cdot m^{-2} \cdot s^{-1}$ of light supplied by fluorescent lamps and an incandescent supplement. Infected plants in all experiments were stunted (including a shortened shoot) with wilted primary leaves.

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After 1–3 days, plants were transferred to a white, three-sided wooden box (96 × 63 × 91 cm) with a 6-mm-thick glass plate top (Fig. 1A). Light in the box was 15.5 $\mu E \cdot m^{-2} \cdot s^{-1}$ and temperature was 23 C. Initial experiments were conducted with unstaked plants because shoots were smaller (free-moving portion of the shoot generally shorter than 8 cm). Plants in later experiments were secured to bamboo stakes, leaving 4–12 cm of the shoot free to move. The vertical distance between the tip of each plant and the glass plate was determined by observing the reflection of a sliding height marker on a ruler set perpendicular to the glass plate and positioned above the tip (5) (Fig. 1A). Horizontal location of the tip was marked with a felt pen as a single dot on the glass plate (Fig. 1A) at 10-min intervals for 210 min or at 20-min intervals for 360 min. Successive points on the glass plate were then connected with straight lines, the pattern was traced on paper, and the distance between points was measured. Speed of moving tips was calculated using values of the horizontal and vertical distances moved, then expressed in millimeters per 10 or 20 min (1,5).

In experiments designed to examine the effect of length of free-moving shoots on circumnutation, plants were grown from seed in a controlled-environment chamber with a light/dark cycle of 15/9 hr (265 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 23 C and relative humidity of about 70%. Eight plants of uniform size were selected, divided into two groups of four plants each, and monitored for 2 days. Each plant in the first group was staked and taped so that the free-moving aerial shoot was 4 cm long at beginning of the first day of measurement. On the second day, shoots were restaked so that the free-moving part was 8 cm long, and on the third day, they were restaked to 12 cm. Shoots of the four plants in the second group were staked to 12 cm the first day, to 8 cm the second day, and to 4 cm on the third day. All eight plants were placed in the three-sided glass-topped box each day for 3 hr before being monitored every 10 min for 3.5 hr. They were returned to the controlled-environment chamber after the last measurement, and the whole procedure was repeated the next day. Because shoots may grow 15–28 cm in a single day, this procedure enabled us to assess the relation of shoot length to speed and to sort out possible age effects.

In the third experiment, a group of 50 greenhouse-grown plants was randomly divided in half. One set of 25 plants was inoculated with *C. flaccumfaciens* 9 or 15 days after planting; the other set served as water-inoculated controls. When disease symptoms became visible, four uniform pairs of plants, each pair consisting of an inoculated plant and a morphologically similar control, were chosen from each group. These plants were staked so that the free-moving shoot in each pair was the same length. Paired plants

were transferred to the controlled-environment chamber for about 1 day, placed in the measuring box for 3 hr, and then monitored every 20 min for 6 hr. Lights were then turned off and plants were left in the box; after 14.5 hr, lights were turned on and plants restaked. Readings were taken every 10 min for 3.5 hr.

RESULTS

Although variations occur among plants, even for a given plant over time, the horizontal trajectory and measurements of speed illustrated in Figures 1B and C are representative of uninoculated

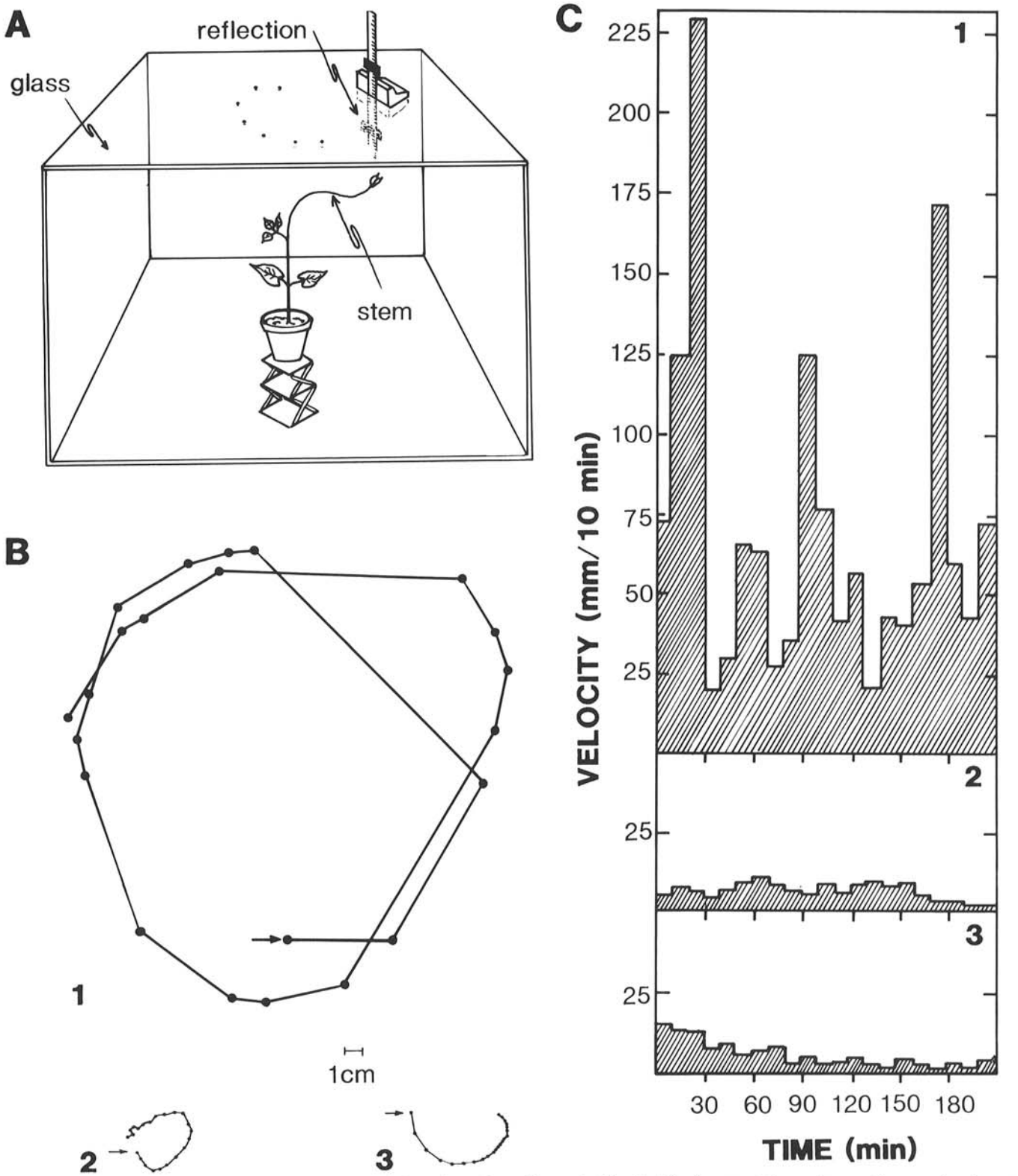

Fig. 1. A, Box for measuring circumnutations of shoots of *Phaseolus vulgaris* 'Kentucky Wonder,' **B,** circumnutation patterns of free-moving stems or trajectory (in a horizontal plane) as viewed and traced onto paper from the upper surface of the glass plate, and **C,** speed of free-moving shoots. Note the difference in trajectory pattern (B) and the speed (C) between the healthy plant shoot (1) and the shoots of the plant invaded by *Corynebacterium flaccumfaciens* (2) or *Xanthomonas campestris* pv. *phaseoli* (3).

TABLE 1. Speed of circumnutation movements of *Phaseolus vulgaris* 'Kentucky Wonder' free-moving shoots on plants infected by bacterial plant pathogens *Xanthomonas campestris* pv. *phaseoli* (Xcp) and *Corynebacterium flaccumfaciens* (Cf)

Plant[a] (no.)	Shoot length[b] (cm)	Pathogen	Speed (mm/10 min) Mean	Range
1	Variable	None[c]	69.7	20–229
1	Variable	Xcp	5.8	1.4–15.0
2	Variable	Xcp	3.7	1.0–8.2
3	Variable	Xcp	1.7	0.0–6.3
4	Variable	Xcp	1.0	0.0–3.0
1	Variable	Cf	6.2	2.8–10.0
2	Variable	Cf	6.1	0.8–18.7
3	Variable	Cf	3.1	0.0–7.3
4	Variable	Cf	1.6	0.0–6.7
5	Variable	Cf	1.5	0.0–3.0
1	8	None	18.3	9.4–29.0
1	8	Xcp	7.5	2.8–11.7
2	8	Xcp	10.9	4.5–12.0
3	8	Xcp	13.2	3.2–28.3
1	8	Cf	2.6	2.2–9.5
2	8	Cf	12.9	3.6–41.0

[a] Unstaked plants, total length of stem and age of plants varied.
[b] Lenth of stem was variable, but in some treatments, the stems were staked to a length of 8 cm before reading.
[c] Only one plant listed for uninoculated control. Speeds of stems on uninoculated plants staked at various legnths are presented in Table 2.

TABLE 2. Speed of free-moving shoot stem circumnutation of *Phaseolus vulgaris* 'Kentucky Wonder' as a function of stem length measured over a 3-day span

Group	Plant (no.)	Mean speed of shoot stem (mm/10 min) (day of measurement and shoot stem length)[a] 1 (4 cm)	2 (8 cm)	3 (12 cm)
A[b]	1	4.0	10.1	54
	2	3.5	3.3	48
	3	5.7	11.4	50
	4	3.0	6.7	64
B[c]		1 (12 cm)	2 (8 cm)	3 (4 cm)
	1	19	8.1	5.0
	2	60	8.7	4.2
	3	32	10.6	6.8
	4	60	12.4	3.5

[a] Stem tip lengths controlled by taping to a stake on each of three successive days.
[b] In group A plants, shoot stems were taped to 4-cm lengths for day 1 measurements, 8-cm lengths for day 2, and 12-cm lengths for day 3.
[c] In group B plants, shoot stems were taped to 12-cm lengths for day 1 measurements, 8-cm lengths for day 2, and 4-cm lengths for day 3.

bean plants. Results from experiments with unstaked plants are illustrated in Figure 1 and Table 1. Both the diameter of the horizontal trajectory and the speed were greatly reduced in plants infected by the bacterial pathogens *X. c.* pv. *phaseoli* and *C. flaccumfaciens*.

Shoots of the diseased plants were generally shorter than those of the healthy plants. To examine effects of shoot length on movements of the shoots, experiments were conducted in which the shoot of an individual plant tip was staked and tied so that it would be either 4, 8, or 12 cm long. Results from this study, in which all three lengths were monitored on single plants (Table 2), illustrate the effects of the free-moving shoot length on speed of tip movement. Faster speeds were associated with the longest free-moving shoots (12 cm).

Four uniform pairs of plants, each pair consisting of one healthy and one diseased plant, were selected from a large population of plants. They were then staked to the length that would provide a suitable free-moving part of comparable length (5–10 cm). The length of each of the two plants was determined. Securing the stem

TABLE 3. Effects of *Corynebacterium flaccumfaciens* infection on circumnutation of *Phaseolus vulgaris* 'Kentucky Wonder' shoots of various lengths over a 2-day span

Time of measurement (day)	Plant (no.)	Treatment	Staked length of shoot stem (cm)	Mean speed of circumnutation (mm/20 min)
1	1	Inoculated	8	14.4
	2	Control	8	67.3
	3	Inoculated	10	70.0
	4	Control	10	108.9
	5	Inoculated	5	3.1
	6	Control	5	10.7
	7	Inoculated	6	2.8
	8	Control	6	12.9
				(mm/10 min)
2	1	Inoculated	10	13.3
	2	Control	10	34.2
	3	Inoculated	10	5.8
	4	Control	10	26.5
	5	Inoculated	6	1.5
	6	Control	6	6.7
	7	Inoculated	7	1.8
	8	Control	7	4.6

to a support, which is often a standard procedure in circumnutation studies (5), assured us that the free-moving parts of the shoots of both the healthy and diseased plants were equal. In all instances, speed of circumnutation for the diseased plant was less than that of the uninoculated plant. This difference was obvious during both days of observation (Table 3).

DISCUSSION

Circumnutation of shoots in certain cultivars of *Phaseolus* are known to be rhythmic (5), but to our knowledge, nothing has heretofore been reported on the effects of disease on the speed of such movements. Stunting is a prominent clinical feature of bean plants infected by *X. c.* pv. *phaseoli* and *C. flaccumfaciens* (7). Length of the free-moving part reflects speed and trajectory of circumnutations (5). However, we were able to evaluate the effects of bacterial infection on circumnutation by securing shoots at specified distances (5–10 cm) to wooden stakes, thus standardizing the shoot lengths of healthy and diseased plants. Considerable variation in the progress of disease was evident. Some plants died early, whereas others continued living with varying magnitudes of stunting. Therefore, it was essential that we compare diseased and healthy plant pairs that were similar in morphology and free-moving shoot length. Both speed and configuration of horizontal trajectory of circumnutation of shoots are affected by disease (Fig. 1, Table 1). The comparative speed of movement may vary greatly from one day to the next (Table 3).

A physiological explanation for the effects of bacterial infection on circumnutation cannot be given because many questions still remain regarding mechanisms of circumnutations per se (2). There are many unanswered questions related to circumnutation, or other movements for that matter, in diseased plants. Bacterial toxins, water stress, and temporal organization are only a few of the many unstudied but possible factors that might affect such movement.

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